PhD értekezés tézisei

Az arzén-kezelés indukálta stressz-folyamatok

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Budapest

2014

Introduction

Besides natural (eg .: South Plains) and anthropogenic arsenic contamination, (eg. cyanide pollution in River Tisza and the Ajka red mud reservoir bursts) restrictions on arsenic content of food and drinking water coming into effect together with accession to the European Union, and with the expiration of the moratorium make our research current. More and more studies are born of purification possibilities of water and soil containing arsenic,the effects of arsenic on plants and the consequences of its entering into the food chain.

The arsenic uptake of plants is significant both in human food and agricultural aspects. The terrestrial and aquatic plants are able to take up arsenic. In addition to the organic arsenic forms, both inorganic arsenic forms may be present in plants, but in different rates,both in different plant species and the various parts of the plant. Therefore,in our studies the following questions are to be answered:what physiological changes can be caused in plants by low (2-10 μM) arsenic concentration found in groundwater of arsenic content or in irrigation water? Where is the primary attack point for arsenic located? Where and how do the symptoms of toxification appear? We assumed that one reason for the toxicity of arsenic may be As (V) ↔As (III) transformation. Is it possible to fend off oxidative stress which siginficantly contributes to arsenic toxicity and how?

Materials and methods

To carry out our experiments,we have decided to use cucumber ,(Cucumis sativus L. cv. Joker 1) for practical purposes, the vegetable that will grow well in a water culture system, under laboratory conditions.

The plants were grown in an air-conditioned plant growth chamber, in ¼ Hoagland modified nutrient solution. Depending on the experimental setup the original phosphate concentration of 250 μM was reduced to 100, 10 and 2 μM. Iron was added to the plants in the form of FeCl3 (10-5 M) or Fe (II) ascorbate (10-5 M) ,to which ascorbic acid (30 μM) was dissolved in FeCl3 (10 μM),which was freshly prepared during the change of nutrient solution. To the experiments arsenite (As [III]), arsenate (As [V]), dimetilarsenate, (DMA), mercury, Fe (II) –ascorbate and silicon were used at various concentrations. Physiological parameters of As-, Hg, Fe (II) ascorbate and Si –treated plants- were compared with data of untreated, (Fe ([CI)] 3) control plants.

To determine deficit of water saturation leaf discs were used,the initial- the turgescent- and the dry weight of which were measured. (Slatyer 1967).
The stomatal conductance, stomatal openess was determined on the bases of the duration of transpiration water permeability by using porometer (Delta T Devices Ltd).
To study bleeding exudate well developed plants were grown in order to have the root pressure constant for one hour. Bleeding exudate was collected in helium atmosphere, into containers cooled below 0 ° C. The weight of the collected bleeding exudate was measured and analyzed by using HPLC-ICP-MS (Andel, 1953).

To verify the change in the conductivity of the root,we used a root placed in distilled water, treated in 4 different temperatures. (Singh et al., 2006).
The extent of lipid peroxidation was determined with a method based on detection of malondialdehyde (MDA) (Gosset et al. 1994).

The ascorbic acid content of the hypocotyl and the root was determined using ascorbate oxidase enzyme, with a spectrophotometer (Shimadzu UV-2101PC) (Knörzer et al. 1996).

The enzyme activity of ascorbic acid oxidase was measured with a spectrophotometer (Shimadzu UV-2101PC) based on the method of Oberbacher and Vines (1963).

Analytical determination of minerals (HPLC-ICP-MS, FIA)

Bleeding exudate was examined with a directly attached,high efficiency,liquid Chromatography, dual focussing inductively coupled plasma mass spectrometer (HPLC-ICP-MS). The total arsenic content of samples was determined with flow techniques (FIA-ICP-MS). Studies were carried out by ELTE Environmental Chemistry Research Group and UNESCO ELTE Trace Element Satellite Centre.

From previous experimental results and from bibliography, we know that the chemical state of arsenic highly influences its toxic effect on plants. Therefore, we were looking for and then developed such examination procedures that made detection and localisation of arsenic forms in the plant possible. Redox processes [As (V) ↔ As (III)], occuring during exploration processes of chemical samples were eliminated,so that we could get a real overview of the localisation of arsenic and the oxidation state of arsenic in the plant.
During the examination procedures based on X-ray fluorescence an intensive synchrotron beam excites the elements of the sample. During the excitation the chemical elements emit

characteristic X photons (X-ray fluorescence), the energy of which uniquely identifies the given mineral.

Mini-X Ag analysis

With the help of the portable X-ray spectrometer measurements were made in the Budapest University of Technology, Institute of Nuclear Technology, Nuclear Energy Department, with the co-operation of Dr. Imre Szalóki Gerényi Anita PhD student (http://www.reak.bme.hu).The effects of arsenic treatments 20, 40, 60, 80 and 100 μM were compared. Measurements were carried out on lyophilized cucumber hypocotyls.

X-ray fluorescence confocal imaging (XRF-Cl)

With the application of confocal (XRF-Cl) measurement technique radiation emitted from a small volume of the sample can be detected,if the sample is in an appropriate position,three-dimensional volumetric analysis can be performed from the measured data. (But Samber 2010a., 2010b.). The XRF-CI (confocal) tests were performed on HASYLAB L beam channel (http://hasylab.desy.de/) with Dr. Imre Szalóki Gerényi Anita and PhD students.

XANES (X-ray Absorption Near Edge)

With XANES analysis technique the chemical status of arsenic was determined in the sample. The XANES examinations were carried out in Grenoble, at the beam channel of the European Synchrotron Radiation Facility (ESRF) (http://www.esrf.eu) and in Hamburg at beam channel of HASYLAB L (http://hasylab.desy.de/).

Sampling and statistical calculations

During our work 2-3 independent experiments were performed in each case, and in an experiment the parallel measurement of 5-10 samples took place . To evaluate measurements we calculated the average and standard deviation (Microsoft Office Exel). Where the evaluation of the data made it necessary, the statistical evaluation was carried out in the Instat 3.0 (GraphPad, Inc.) program, one-way ANOVA, Tukey-Kramer post hoc test.

Results and conclusions

1.Similarly to the so-far-studied plants in the bibliography cucumber also took up arsenate,arsenite and dimethyl-arsenate from the nutrient solution. It proved to be a good test plant because besides its being easily growable it reacted sensivitely to arsenic treatments in low concentration,so that we could demonstrate that arsenic presence in the nutrient solution primarily affects water supply of the plant and this effect depends on the developmental stage of the cucumber.

2. As (V) is taken up through phosphate transporter also in cucumber. The surplus of phosphate reduces the uptake of As (V), while it did not affect the uptake of As (III. As (III), according to bibliography data, enters the plant through the aquaporin channels. In rice and maize it is the Silicon transporters are responsible for the uptake of As (III) both in case of rice and maize, they transport arsenite as well as silicon to the stele with high efficiency. In our experiments we could not demonstrate the positive effect of the silicon in cucumber neither through elimination of the radical formation, nor its competition in the transport of As (III).

3. Arsenic primary point of attack is the root of cucumber, as As (V) is reduced to be → As (III) and consequently free radicals are formed, oxidative stress occurs, leading to lipid peroxidation and the damage of membranes. Based on our studies extent of arsenic toxicity was affected by the developmental stage of the plant. In the young plant it induced growth inhibition and aging processes. Due to the stressor adventitive roots were formed. In hypocotyl it increased concentration of ascorbic acid and activity of ascorbic acid oxidase. All these factors have ensured the survival of young plants. In the plant with one leaf (sensitive period) As (V) treatment caused turgor loss of the root and shoot, damage of the water household and membrane desorganisation. The sensitive period - during the intensive growth of the leaves - the antioxidant defense system could not prevent oxidative degradation . The concentration of ascorbic acid and the activity of ascorbic acid oxidase was barely measurable. As a result of the treatment the plant has got into its depletion phase. In case of plants with 2-3 leaves and developed roots As (V) treatment in addition to the slight growth inhibition caused partial root turgor loss, however,the water retaining capacity of the shoot was no longer affected. The concentration of ascorbic acid and ascorbic acid oxidase activity of the plants treated approximated the control values.

4. With addition of Fe (II) ascorbate to the nutrient solution, no sensitive period in plants was detected,hypocotyl remained turgerscent, free radicals formed during the As (V) ↔ As (III) transformation were eliminated.

5. The effect of arsenic on aquaporins may also be behind the water supply disorders . It can be assumed that during the phosphorylation↔dephosphorylation As (V) may substitute phosphate. As (V) - and Hg (II) treatment inhibited the growth of root and shoot, the

transpiration of water saturation pressure deficit and root formation approxiamtely equally. Results suggest the role of aquaporins.

6. In our work we have successfully applied X-ray fluorescence micro-tomography on biological samples, the X-ray confocal imaging method and the XANES technique. We eliminated redox processes (As (V) ↔ As (III)) which were occuring during exploration processes of chemical sample, so that we could get a real overview of the location and oxidation state of arsenic in the plant. Cucumber takes up,converts and translocates arsenic compounds. In case of plants treated with arsenate the XANES tests demonstrated that the oxidation state of arsenic varies in different parts of plants. Based on the spectra, in roots treated with arsenic As (V), in the hypocotyl As (V) and As (III) presence were detected (al Silversmit et. 2009). In the xylem sap the predominant form of arsenic was the As (III) detected with HPLC-ICP-MS method. With fluorescent X-ray micro-tomography it was demonstrated that arsenic masses up mainly in the epidermis of hypocotyl, in parenchyma cells with thicker cell walls and in vascular tissues. High arsenic concentration in the epidermis appears to be one way of protection. From arsenic forms (As (V) and As (III)) found in hypocotil, it can be concluded that arsenic transformation can happen not only in the roots, but also in hypocotyl, which can cause oxidative stress.

Based on our results we concluded the following:

i.,

Arsenic primarily affects the water household of the plant. The effect will depend on the developmental stage of the cucumber.

ii.,

In the roots of the cucumber arsenate is reduced into arsenite, the dominant arsenic form is arsenite in the xylem sap. With arsenate treatment arsenate occurs besides arsenite in the hypocotyl. During reduction of arsenate into arsenite free radicals are formed, oxidative stress occurs, leading to lipid peroxidation and the damage of membranes.

iii.,

Fe (II) ascorbate added to the nutrient solution reduces the toxic effect of the arsenic treatment. The hypocotyl and the rest of the shoot remains turgerscent, dependence on the state of plant developmental stage or the sensitive period is not detected.

iv.,

During the intensive growth of the first leaf Fe ascorbate treatment significantly increases the concentration of ascorbic acid and ascorbic acid oxidase.

v.,

As (V) - and Hg (II) treatment approximately equally affects the growth and water household of cucumber. Results suggest the role of aquaporins.

vi.,

Arsenic can be found in the root tissues,in the epidermis of hypocotyl, in parenchyma cells with thicker cell walls and in the vascular tissues. Higher arsenic concentrations in the epidermis can be one way of protection.

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